



Wolbachia-based mosquito management for Aedes spp.

Milutin Djurickovic – Intro to *Wolbachia* / Product Characterization

Jeannine Kausch – Regulatory Aspects

John Kough – Human Health Considerations

Shannon Borges – Environmental Risk Assessment

Chris A. Wozniak – Oxitec GE Mosquito

Biopesticides and Pollution Prevention Division

Office of Pesticide Programs

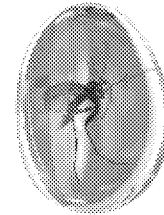
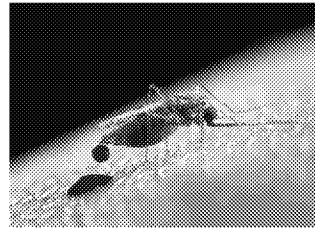
United States Environmental Protection Agency¹



Mosquito Basics

- Females bite / Males do not
- 3500 mosquito species

- Only 9 species bite
- Females much larger than males
- 220 million years – not much variation



United States Environmental Protection Agency²



Mosquitoes

- *Ae. aegypti* – Yellow fever mosquito
- *Ae. albopictus* – Asian Tiger Mosquito – day time biter
- *Albopictus* displacing *Aegypti*
- *Can transfer disease to progeny – but less common*



United States Environmental Protection Agency³



***Ae. aegypti* distribution**

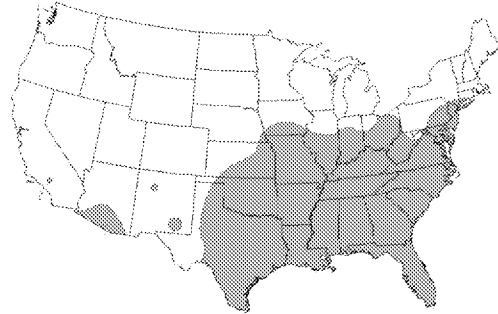


<http://www.cdc.gov/cdcgrandrounds/pdf/archives/2015/may2015.pdf>

United States Environmental Protection Agency⁴



***Ae. albopictus* distribution**



<http://www.cdc.gov/cdcgrandrounds/pdf/archives/2015/may2015.pdf>

United States Environmental Protection Agency⁵



Aegypti vs. Albopictus

Aegypti – Yellow fever mosquito

- ❖ Zika transmitter
- ❖ Yellow fever – Vaccine 1930's
- ❖ Chikungunya
- ❖ Strongest Dengue Transmitter
- ❖ Thrives in Urban Areas – mostly bites people
- ❖ Brought to new world during colonization

Albopictus – Asian tiger mosquito

- 1985 Texas
- Dengue – Break bone fever
- Eastern Equine Encephalitis
- Bites man, wild animals, and domestic animals
- Cache Valley virus
- St. Louis and LaCrosse Encephalitis



Wolbachia biocontrol

- *Wolbachia* – intracellular microbe
- Naturally Present in *Culex* and *Albopictus* mosquitoes species and fruit fly
- Endosymbiont – lives inside exclusively
- Present in ~60% of all insect species
- Maternally inherited and Horizontally
- Not naturally present in *Aegypti*

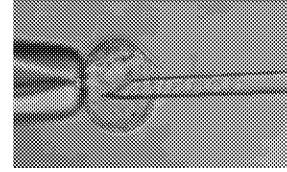


<http://www.cdc.gov/cdcgrandrounds/pdf/archives/2015/may2015.pdf>

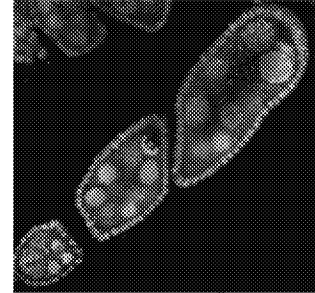
United States Environmental Protection Agency⁷



Wolbachia pipientis



- Extracted from *Culex* mosquito and microinjected into *Albopictus* and *Aegypti*
- Microinjection and from *Albopictus* to *Aegypti*
- Rear in lab



United States Environmental Protection Agency

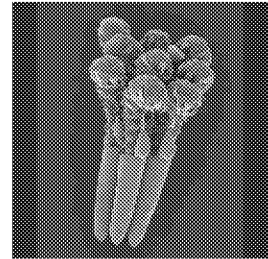
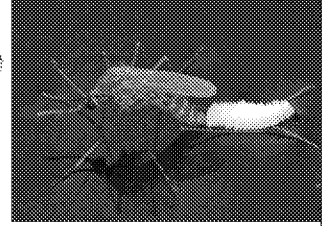


***Wolbachia* Biocontrol**

- ☐ Release males harboring *Wolbachia* into a population
- ☐ Mate with wild females with no *Wolbachia*, eggs don't hatch
- ☐ Females with *Wolbachia* mate with males who don't – they hatch

- ☐ Same *Wolbachia* strain hatch – different don't hatch

- ☐ Problem – sorting males and females for release
- ☐ Horizontal transfer between species – How? Is it recent?
- ☐ Risk – Parthenogenesis? (Females produces females without mating)
- ☐ Kill Males? Feminization?

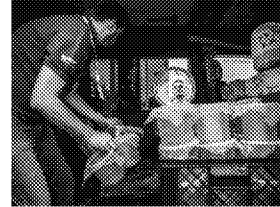
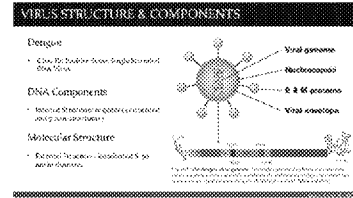


United States Environmental Protection Agency⁹



Eliminate Dengue

- *Wolbachia* establishment — supported by Gates Foundation
- Release Males and Females with *Wolbachia*
- Presence of *Wolbachia* in *Aegypti* lowers virus multiplication
- Result — Less Dengue transmitted
- Success — Australia, SE Asia
- Brazil — problems - coordination

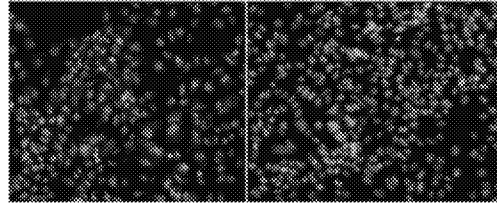


United States Environmental Protection Agency



Eliminate Dengue

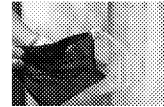
- ☐ FDA will most likely regulate
- ☐ Animal Drug – lessens transmission
- ☐ But may be minimally Pesticidal and Animal Drug
- ☐ May shorten longevity and fertility



United States Environmental Protection Agency¹¹



***Experimental Use Permits In General
(FIFRA § 5 & 40 CFR Part 172)***



- Allow experimental testing of new pesticides and new uses of registered pesticides to accumulate information to support an application under FIFRA section 3
- Required if pesticide field tests are conducted on 10 acres or more of land or 1 acre or more of water
- Certain things must be provided to EPA to support an application for an experimental use permit:
 - ✓ For microbial pesticides, data/information described 40 CFR § 158.2171 through § 158.2174 (e.g., toxicological data/information)
 - ✓ Description of experimental program, including items like amount of pesticide to be used; pest organism involved; and testing locations, dates, and purpose/objectives
 - ✓ Labeling



Wolbachia Experimental Use Permits



89668-EUP-1

- Issued to MosquitoMate, Inc. on July 26, 2013; amended and extended on June 26, 2014
- *Wolbachia pipientis*, ZAP Strain in male *Aedes albopictus*
- 2013 – 2015 in Los Angeles Co., CA; Manatee Co., Florida; and Fayette Co., Kentucky
- 2013 – 2016 in Suffolk Co., New York
- 16,983 acres involved
 - Release site and monitoring acreage
- 127,200,000 *Wolbachia*-infected mosquitoes approved for release
- Generation of product performance data
 - Mosquito adults and eggs to be monitored in treatment and control areas to evaluate whether there is increased frequency of eggs failing to hatch and/or significant reduction in population size in treatment areas

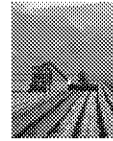




Wolbachia Experimental Use Permits

88877-EUP-2

- Issued to the University of Kentucky on October 15, 2015
- *Wolbachia pipientis*, wAlbB Strain in male *Aedes aegypti*
- 2015 – 2016 in Fresno County, California over 840 acres
 - Release site and monitoring acreage
- 2,400,000 *Wolbachia*-infected mosquitoes approved for release over 6-month timeframe (100,000 per week)
- Generation of product performance data
 - Mosquito adults and eggs to be monitored in treatment and control sites to evaluate whether there is increased frequency of eggs failing to hatch and/or a significant reduction in population size in treatment areas





HUMAN HEALTH CONSIDERATIONS

Wolbachia pipientis first described from *Culex pipientis* in 1936

Related to other intracellular parasites like *Rickettsia*, *Ehrlichia*

Found in numerous forms of life: many arthropods, nematodes

No separate species; now treated as genetic clades

Six clades: A,B,E, F Arthropods; C and D in Nematodes



Major Effects on Hosts

Can range from mutualism to parasitism
Often found associated with chromosomes

Male killing

Feminization

Parthenogenesis

Cytoplasmic incompatibility

Obligate symbiont in nematodes; associated with adverse reaction
in River Blindness, Filarial Diseases



Wolbachia Mosquitoes

Infection with *Wolbachia* to yield unusual phenotypes

Can be employed similar to Male Sterile Technology

Do NOT release females: establish new population

But...

Risk of bite from *Wolbachia* female?

Pre-existing populations of *Wolbachia* mosquitoes

No indication that *Wolbachia* found in salivary glands

No immune reaction to *Wolbachia* from mosquito bites



Role of *Wolbachia* in Human disease?

Nematode diseases shown to be controlled by doxycycline
Obligate symbiont *Wolbachia* in these nematodes
Some implication that release of *Wolbachia* with worm death
may induce inflammatory response triggering pathological effects

So...

Different genetic clade in mosquito versus nematode
Exposure differences: Bite versus systemic worm
No reproduction of *Wolbachia* in mammals
No anti-*Wolbachia* antibody produced



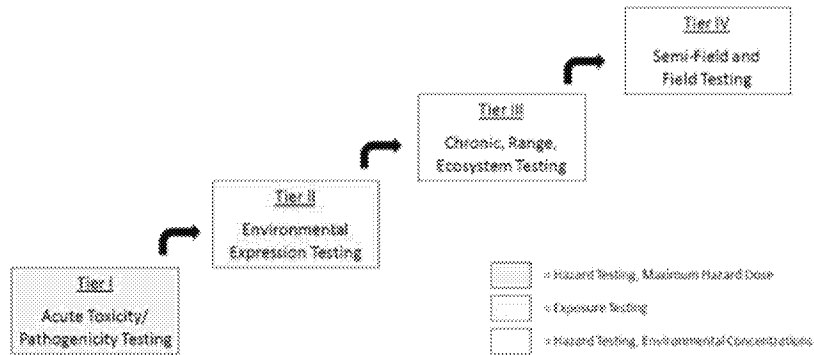
Wolbachia pipientis **Ecological Risk Assessment**

Microbial Pesticides Branch

United States Environmental Protection Agency¹⁹



Microbial Pesticide Nontarget Data Requirements





Nontarget Data Requirements – Tier I

Data Requirement	Guideline	Study Performed	When Required
Avian Oral Toxicity	885.4050	5-day oral exp., 30-day observation	All outdoor applications
Avian Inhalation Tox./Path.	885.4100	5-day intranasal/intratracheal exp., 30-day observation	When nature of pesticide indicates toxicity or pathogenicity to birds
Wild Mammal Tox./Path.	885.4150	5-day oral/intranasal exp., 30-day observation	Case-by-case; if toxicity testing (158.2140) is not adequate/appropriate
Freshwater Fish Tox./Path.	885.4200	Oral/water column exp., 30 day observation	Aquatic or non-residential terrestrial outdoor uses; generally, if significant exposure is expected
Freshwater Invertebrate Tox./Path.	885.4240	Water column exp./30-day observation	Aquatic or non-residential terrestrial outdoor uses; generally, if significant exposure is expected
Estuarine/Marine Fish & Invertebrate Testing	885.4280	Oral/water column exp. (fish), oral exp. (invertebrates), 30 day observation	Only required when significant exposure is expected
Nontarget Plant Testing	885.4300	Exp. route determined by use, observation through harvest or at regular intervals (perennials)	If microbial pesticide is taxonomically related to known plant pathogen
Nontarget Insect Testing	885.4340	Usually oral exp., 30 days or 20% control mortality; 3 species tested	Required when microbial pesticide controls insects by infectivity; may be required when insect concerns exist
Honey Bee Testing	885.4380	Adult or larvae, oral exposure, 30-day obs.	All outdoor applications, may be required for greenhouse uses



Considerations for Nontarget Risk

- Can't be cultured on its own
 - Typical nontarget testing not really possible
- *Wolbachia* is widespread in insects
 - Nontarget organisms are currently exposed
- Exposure to this *Wolbachia* is limited to released adult males
 - Limited nontarget exposure



Primary Focus for Nontarget Risk

- Risk concerns focused on *Wolbachia*'s characteristic effects
- Horizontal transmission to other insects
 - Occurrence on evolutionary time scale
 - Possible cause of new infections
 - Difficult to achieve in the lab
- Impact of mosquito reduction on nontarget food sources
 - *Wolbachia* targets one species
 - Target mosquito species are invasive



SUMMARY

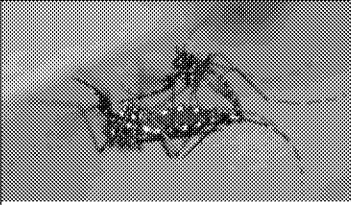
- *Wolbachia* affords some potential for mosquito population suppression through cytoplasmic incompatibility
- Overall risks to human health and the environment are considered low
- Specificity of *Wolbachia*-based management is key to reduced risk paradigm (reduced insecticide use follows)
- We will need to consider this technology as part of an overall integrated approach to managing disease vectors

United States Environmental Protection Agency²⁴



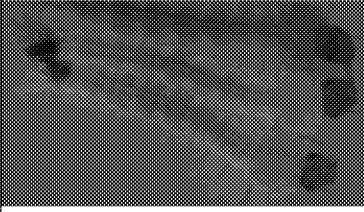
Oversight of Genetically Engineered Male-Sterile Mosquitoes

- FDA-Center for Veterinary Medicine - Investigational New Animal Drug (INAD) – Oxitec OX513A
- Definition of INAD is quite broad
- National Environmental Policy Act (NEPA)
- Environmental Assessment and Finding of No Significant Impact are out for public comment



Planned Florida Release

- Oxitec working with the Florida Keys Mosquito Control District, Key Haven, Monroe County, Florida
- Ovitraps and adult sentinel traps
- Suppression of population of *Aedes aegypti* is the target – not disease transmission



Oxitec OX513A

Aedes aegypti

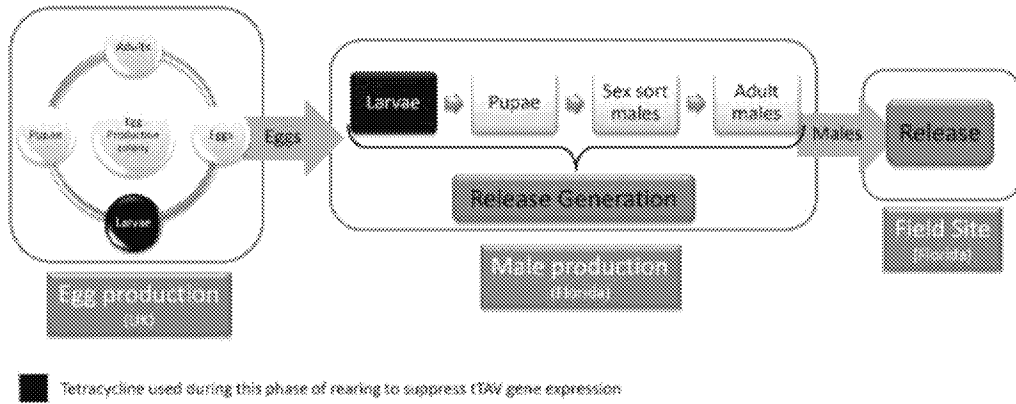
Genetic Sterility

- tTAV – Tetracycline responsive element
 - *insect-optimized tetracycline repressible transactivator protein*
- *tetOx7* – *non-coding binding site for tTAV*
- dsRed2 – *Discosoma Red* Fluorescent protein marker
- In absence of tetracycline, leads to transcriptional squelching – gene expression disrupted

United States Environmental Protection Agency ²⁷



Figure 4: A Schematic of the Production Processes for Producing Males for Release.



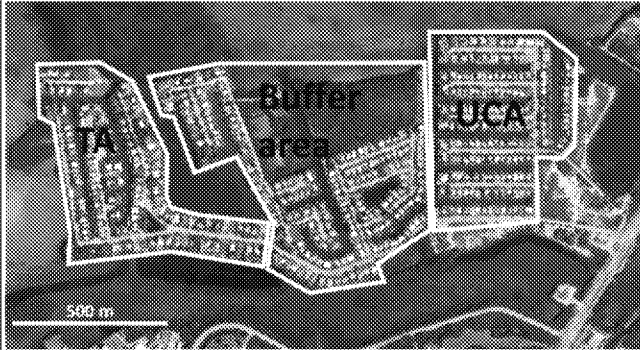


Oxitec Male Sterile-based mosquito management for Aedes spp.

- Male mosquitoes released 2-3X / wk
- Mate with wild-type females, but offspring do not develop beyond larval stages
- Sites to be monitored and dsRed2 marker used to discern which mosquitoes arise from OX513A release
- > 50% suppression of local population is goal



Figure 10 Proposed Trial Area on Key Haven.



Proposed site for investigational release of OX513A mosquitoes. Areas identified are Treated (TA), Buffer, and Untreated Control Areas (UCA), respectively.

Websites for info on Oxitec OX513A

- <http://www.oxitec.com/health/florida-keys-project/>
- <http://www.fda.gov/AnimalVeterinary/DevelopmentApprovalProcess/GeneticEngineering/GeneticallyEngineeredAnimals/ucm446529.htm>

United States Environmental Protection Agency ³⁰